Claim 22 of the present application is drawn to a unique method for reducing the shedding of *E. coli* O157:H7 in an animal through the administration of a vaccine composition containing the whole cells of inactivated or killed *E. coli* O157:H7 in admixture with a metabolizable oil adjuvant. Dependent Claim 23 is drawn to a method for reducing the shedding of *E. coli* O157:H7 that provides additionally administering *Lactobacillus acidophilus* or a neomycin medicated feed supplement to the animal being vaccinated.

Food poisoning with the potentially deadly strain of *E. coli* O157:H7 causes a serious health risk. *E. coli* O157:H7 shed in the feces of cattle has been linked to hemorrhagic colitis and serious kidney damage in humans. Consumption of beef products from contaminated cattle is one source of infection. Another outbreak of *E. coli* O157:H7 in humans was recently traced to tainted spinach where cattle waste from a near-by ranch polluting the irrigation water in the spinach fields was the likely source of the bacterial strain. To get to the source and reduce the prevalence of *E. coli* O157:H7 in the environment, control management at the farm level requires that the *E. coli* contaminant in bovine feces be lessened by decreasing the number of cattle shedding the pathogen in feces. There is an art-recognized need for a viable vaccine that provides improved protection against *E. coli* O157:H7 and reduces the incidence of outbreaks by reducing the shedding of the *E. coli* O157:H7 strain in the feces of cattle, the known carrier and source of food and drinking water contamination. Surprisingly, the new vaccine of the present invention solves the long-felt but unresolved need by significantly reducing shedding in cattle and protecting humans against *E. coli* O157:H7 infection. From the below remarks, the Examiner will appreciate that the benefits of Applicants' vaccine formulation to infuse active immunity in the cattle against shedding and to obtain strong antibody titers that prevent colonization of the *E. coli* O157:H7 as well as provide bactericidal effect are neither taught nor suggested in the art.

Turning to the Office action, Claims 22 and 23 are rejected under § 103(a) as being unpatentable over Doyle *et al.* in view of Clancy *et al.* and further in view of the Sigma Catalog as explained on pages 3 and 4 of the action. Applicants respectfully traverse the rejection.

To establish a *prima facie* case of obviousness, the guidelines of M.P.E.P. § 706.02(j) and the case law provide three basic criteria: (1) There must be some suggestion or motivation to modify the reference or to combine the reference teachings; (2) there must be a reasonable expectation of success; and (3) the combined references must teach or suggest all claim limitations.

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163 DELAWARE AVENUE - SUITE 212 • DELMAR, NEW YORK 12054 • TELEPHONE: (518) 475-0611

Examining what the collective art fairly teaches to the ordinary practitioner, it is clear that the practitioner would not arrive at the claimed invention. The art totally fails to provide any suggestion or motivation of doing what the inventors have done.

Doyle *et al.* teach the administration of dominant probiotic bacteria, namely, the specific strains of *E. coli* 271, *E. coli* 786 and *E. coli* 797, to a ruminant animal to prevent and treat the carriage of *E. coli* O157:H7. Patentees indicate that animals known to be shedding *E. coli* O157:H7 in feces are suitable candidates for treatment with their dominant probiotic bacteria (see col. 5, lines 62-65) yet Fig. 2 shows that shedding of *E. coli* O157:H7 in feces at various levels was continuous throughout their experiment and Fig. 6 demonstrates that continuous shedding of *E. coli* O157:H7 at various levels in the feces of 11 of 12 calves occurred throughout the study. Doyle *et al.* suggest that feeding non-pathogenic probiotic bacteria to cattle reduces the carriage of the harmful *E. coli* O157:H7 bacteria as a consequence of the competition in the rumen of the animal. However, Doyle *et al.* do not imply that probiotic bacteria can act in any way, shape or form as a vaccine to reduce shedding.

Indeed, Doyle *et al.* expressly teach: "Vaccines are not likely to be effective in reducing the amount of *E. coli* O157:H7 carried and shed by cattle" (col. 2, lines 2-3). Although Doyle *et al.* say that vaccination has been the traditional approach to protecting cattle from carriage of harmful bacteria, they explain that there is difficulty in vaccinating cattle against *E. coli* O157:H7 because the strain does not adhere to or attach to colon tissue and does not infect cattle. As a consequence, Doyle *et al.* use the non-pathogenic dominant probiotic bacteria to reduce localization of *E. coli* O157:H7 in the rumen since they believe that the rumen is the most important site for long-term carriage of *E. coli* O157:H7, and may serve as the source of bacteria found in the colon.

In effect, Doyle *et al.* provide a negative teaching away from Applicants' method for reducing shedding that is uniquely achieved through vaccination of cattle. Plain and simple, based on Doyle *et al.*, there is no question that one of ordinary skill in the art would be deterred from vaccinating cattle and accomplishing what Applicants have done. The practitioner would have no reasonable expectation of success in reducing shedding of *E. coli* O157:H7 through the administration of Applicants' vaccine composition and the stimulation of a strong immune response. It is quite unexpected, therefore, that Applicants demonstrated a significant reduction

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of pathogen prevalence in the hide and fecal samples of vaccinated cattle in the working Example 3 on page 13 of the application.

In light of the negative teaching of Doyle *et al.* and the unexpected results of the present invention, there is no reason in the art to motivate the practitioner to combine the disclosures of the cited references and modify the teachings sufficiently to arrive at the claimed method.

Clancy *et al.* relate specifically to compositions and vaccines useful for prophylactic or therapeutic treatment of mucosal infections in the respiratory tract. The mucosally administrable composition of Clancy *et al.* comprise one or more antigens derived from at least one microorganism which is capable of causing an infection at a mucosal surface and a probiotic, that is, an adjuvant capable of inducing a Th1 cellular immune response, wherein the adjuvant is not derived from a microorganism capable of causing infection at a mucosal surface. The reference teaches that the antigen is derived from a bacterium, a fungus or a virus, but only describes those antigens that are respiratory tract pathogens, *i.e.*, those that normally colonize the respiratory tract (specifically, NTHi, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae, Staphylococcus albus* and *Staphylococcus aureus*).

Although the reference broadly suggests that the vaccine makes use of any mucosal pathogen and any mucosal surface including the intestinal tract, Clancy *et al.* purely teach respiratory tract vaccines that may be administered to any mucosal surface and have the desired effect because of the common mucosal immune system, showing, for example, the immunization of rats against non-typeable *H. influenzae* (NTHi) by an intra-lumenal (IL) injection (into the lumen of the small intestine). Clancy *et al.* do not describe, exemplify or suggest any antigens that colonize the intestinal tract, let alone *E. coli* O157:H7. Besides, Doyle *et al.* explicitly state that *E. coli* O157:H7 does not adhere to or attach to colon tissue; and it is well known that *E. coli* O157:H7 does not infect cattle. As such, there is absolutely no reason to predict that the mucosally administrable composition of Clancy *et al.* could work with the whole cells of inactivated or killed *E. coli* O157:H7 to reduce shedding in the feces of cattle.

In terms of Applicants' unique vaccine formulation, the combined art does not teach or suggest using a metabolizable oil adjuvant in a vaccine containing whole cells of *E. coli* O157:H7. Clancy *et al.* merely disclose that additional known conventional adjuvants may be included in their respiratory tract vaccine. Since Clancy *et al.* do not even suggest including a

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163 DELAWARE AVENUE - SUITE 212 • DELMAR, NEW YORK 12054 • TELEPHONE: (518) 475-0611

specific adjuvant such as the metabolizable oil in a respiratory tract vaccine, such a bare and limited disclosure certainly does not imply using the metabolizable oil adjuvant elsewhere.

The reference to the Sigma catalog also fails to suggest using the metabolizable oil adjuvant in the present case. Page 1472 of the Sigma catalog merely shows that a metabolizable oil (squalene) is commercially available as one of three components in the TiterMax Gold and Classic Adjuvants consisting of a block copolymer, squalene and a sorbitan monooleate (Gold Adjuvant) or a block copolymer, squalene and a microparticulate stabilizer (Classic Adjuvant). A generic list of adjuvants from one chemical supplier's catalog does not provide any teaching of which particular adjuvant can be used in concert with which antigen for what results. The sheer number of adjuvants that are commercially available from different sources is enormous. Without some direction as to exact combinations, a long list of adjuvants does not describe Applicants' claimed composition or vaccine. Unless there is some teaching in Doyle *et al.* or Clancy *et al.* to give motivation to find a metabolizable oil on this specific page of the Sigma catalog (and not consider the present claims through impermissible hindsight vision), one of ordinary skill in the art would have no reason to look at this catalog, much less select a specific adjuvant comprising the claim-recited metabolizable oil. The fact that the ordinary practitioner needs to pick and choose among a huge variety of options means that the reference does not render the present invention obvious.

Moreover, the unique metabolizable oil adjuvant in Applicants' vaccine composition provides beneficial and unexpected results over those seen with conventional adjuvants that are not taught in the art. Shown in working Example 2 of the application, the metabolizable oil in the vaccine of the present invention (Group 7) demonstrated significant improvements over the standard vehicle (aluminum hydroxide) used in the comparative vaccine of Group 6. Quite unexpectedly, the vaccine formulation of the invention provided the greatest overall serological titers and the best improvements in immunity; and, equally surprising under the circumstances, the animals displayed minimal, normal reactions at the vaccine administration sites.

As previously noted in the record, it was not anticipated that the vaccine of the invention would be safe on administration. With all vaccinations, a little lump is expected when the active ingredient is released slowly from the site of depot administration. Typically, vaccines that give a higher immune response cause a greater reaction. Because a severe lump develops from

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163 DELAWARE AVENUE - SUITE 212 • DELMAR, NEW YORK 12054 • TELEPHONE: (518) 475-0611

vaccines with significantly higher immunogenic responses, it was initially thought that the claimed vaccine composition would cause a greater adverse reaction. It could not be predicted that the size of the reaction lump of the vaccine of the invention would be the same as the traditional vaccine and no major reaction would be observed. Despite the higher immune response, the results unexpectedly demonstrated that the vaccine containing the metabolizable oil was safe for administration to cattle.

Taking the invention as a whole, it is clear that one of ordinary skill in the art would not arrive at the claimed invention from the teachings of the combined references. First of all, Doyle *et al.* and Clancy *et al.* do not teach methods for reducing the shedding of *E. coli* O157:H7 in an animal through active immunity, *i.e.*, inoculating cattle with an effective vaccine composition. Secondly, it cannot be inferred from either of these two references that a vaccine containing inactivated or killed whole cells of *E. coli* O157:H7 would work against *E. coli* O157:H7. Based on the negative teachings of Doyle *et al.* and the limited respiratory tract vaccine of Clancy *et al.*, the practitioner could not anticipate being able to achieve a superior immune response to the vaccine formulation comprising whole cells of *E. coli* O157:H7 and a metabolizable oil adjuvant. The combined references simply fail to teach or suggest all claim limitations of Applicants' method.

The above showing of the references' flaws and the objective evidence of the superior activity of the vaccine of the present invention sufficiently refutes the holding of obviousness.

The Examiner separately rejects Claim 23 under § 103(a) as being unpatentable over Doyle *et al.* in view of Clancy *et al.* and further in view of the Sigma Catalog as applied to Claims 22 and 23 above, and further in view of Molly *et al.*, as set forth on pages 5 and 6 of the Office action. Applicants respectfully traverse the rejection.

Molly *et al.* relate to a growth promoter composition suitable for animals that comprises a fungus and at least one growth-promoting component comprising organic acids, inorganic acids, animal feed antibiotics, conventional growth promoters or plant extracts. The fungus, which is a critical component of the growth promoter composition, is never omitted from any composition described by Molly *et al.* While they disclose that one aspect of their invention involves feeding the growth promoter composition to an animal and inducing changes in the microbial ecosystem in the gastrointestinal tract of the animal, this method for improving the gastrointestinal tract

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163 DELAWARE AVENUE - SUITE 212 • DELMAR, NEW YORK 12054 • TELEPHONE: (518) 475-0611

would mandate that the entire composition be given to the animal, including the fungus taught by Molly *et al.* as an essential feature.

Although the reference generically discloses that an undesired enteric pathogen could be *Escherichia* among a large list of other enteric pathogens and generically identifies neomycin as an animal feed antibiotic that could be added to the fungus of the growth promoter composition, Molly *et al.* purely suggest that neomycin be used in combination with the fungus to improve the gastrointestinal microbial ecosystem by suppressing pathogens in the gastrointestinal tract of the animals. However, there are no specific formulations or examples that contain neomycin. Rather, exemplification in Molly *et al.* is limited to showing the influence of *Lentinus edodes* in combination with a growth-promoting component on the growth and feed conversion ratio (FCR) of chickens and pigs.

Additionally, Molly *et al.* indicate that while FCR can be lowered by influencing the bio-regulatory process through administering traditional antimicrobials or feed antibiotics, antibiotic therapy has the disadvantage in that it results in the destruction of the intestinal microflora ([0005]). Reading the entire disclosure, it is plain to see that Molly *et al.* do not promote the use of an animal feed antibiotic such as neomycin in the absence of fungus as it will have an adverse effect on the animal.

Without question, the combined references do not teach or suggest all of the limitations of Claim 23. Neither Doyle *et al.* nor Clancy *et al.* teach a vaccine containing whole cells of *E. coli* O157:H7. There is no suggestion or motivation in either reference to combine their teachings with the Sigma catalog and make a precise selection of only one adjuvant, the metabolizable oil, out of numerous commercially available adjuvants from Sigma and elsewhere. Certainly, Molly *et al.* do not add the motivation to select and use neomycin in combination with a vaccine for reducing shedding of *E. coli* O157:H7. Since the references do not propose Applicants' method and they do not provide a basis to expect that the claimed method would be successful in reducing the shedding of *E. coli* O157:H7, this rejection cannot be sustained.

Claim 22 is also separately rejected under § 103(a) as being unpatentable over Johnson *et al.* in view of the Sigma Catalog as detailed on pages 6 and 7 of the Office action. Applicants respectfully traverse the rejection for the following reasons.

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163 DELAWARE AVENUE - SUITE 212 • DELMAR, NEW YORK 12054 • TELEPHONE: (518) 475-0611

Johnson *et al.* describe inoculating dairy calves with a vaccine containing inactivated *E. coli* O157:H7, inactivated verotoxin 2 and intimin O157; and then challenging the calves with an oral antibiotic-resistant strain of *E. coli* O157:H7. The authors found little difference between vaccinated and control calves. Despite the strong and sustained antibody responses to the vaccination, vaccinated calves did not shed significantly fewer bacteria than the control calves. There was little difference shown between vaccinated and control calves both in the levels and the duration of shedding. Johnson *et al.* conclude that infection of naturally reared calves by *E. coli* O157:H7 is unlikely to be controlled by immune responses induced by parenterally administered inactivated bacterins.

First and most importantly, Johnson *et al.* failed to accomplish what Applicants have done. They did not reduce shedding of *E. coli* O157:H7 in the feces of cattle. Secondly, Johnson *et al.* disclose a totally different vaccine composition containing inactivated *E. coli* O157:H7, inactivated verotoxin 2 and intimin O157, which does not suggest Applicants' efficacious vaccine formulation. Verotoxin 2 is a known Shiga toxin produced by *E. coli*. The protein is made up of two subunits: one is responsible for toxic action and the other, for binding to a specific cell type. Verotoxin requires highly specific receptors on the host cells' surface to attach and enter the cell. Cattle do not carry these receptors and, consequently, they shed the bacteria in their feces without being infected by the bacteria. Intimin O157, extracted from the outer membrane of *E. coli* O157:H7, is a bacterial protein that permits the *E. coli* to adhere to the host's intestinal cell walls. The bacteria require intimin to colonize their host, attach themselves to intestinal tissue and cause human disease. Much research has been placed on developing vaccines that prevent the transmission of the bacterial protein intimin to the host cell. It makes sense, therefore, that Johnson *et al.* would attempt to use a vaccine that employs intimin O157 along with the Shiga toxin to produce antibodies against *E. coli* O157:H7. Particularly where verotoxin 2 and intimin O157 are art-recognized as essential for bacterial activity, Johnson *et al.* clearly do not teach or propose using the whole cells of *E. coli* O157:H7 in the absence of verotoxin 2 and intimin O157. One of ordinary skill in the art, seeing the failed experiment of Johnson *et al.* in light of common knowledge about verotoxin 2 and intimin O157, would have no motivation to make and use a vaccine containing inactivated or killed whole cells of *E. coli* O157:H7 as the only antigen.

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163 DELAWARE AVENUE - SUITE 212 • DELMAR, NEW YORK 12054 • TELEPHONE: (518) 475-0611

In view of their failure and unacceptable results, Johnson *et al.* teach away from discovering a viable vaccine composition containing inactivated *E. coli* O157:H7. The practitioner would have no reasonable expectation of success in reducing shedding of *E. coli* O157:H7 by a vaccination approach. The practitioner would be discouraged from making and using the whole cells of inactivated or killed *E. coli* O157:H7. Based on Johnson *et al.*, one would not predict being able to control *E. coli* O157:H7 by immune responses induced by parenterally administered inactivated bacterins.

It is unexpected that the claim-recited vaccine composition can stimulate a cell-mediated immune response and be effective in reducing shedding to a significant degree. Working Example 3 on page 13 of the application demonstrates that the vaccine reduced pathogen prevalence by 31.1% in fecal samples. The animal study published by the National Cattlemen's Beef Association, previously supplied to the Office, shows how the percent of positive *E. coli* O157:H7 isolates in the fecal sample of the control (45.8%) was substantially reduced in the vaccinated group (14.7%). The percent of positive *E. coli* O157:H7 isolates on the hide sample of the control (40.3%) was also considerably reduced in the vaccinated group (20.0%). Applicants' dramatic results provide objective evidence of non-obviousness and patentability of the present method for reducing the shedding of *E. coli* O157:H7.

In view of the foregoing remarks, it is respectfully asked that the art rejections of the pending claims be withdrawn and the application be allowed. Favorable treatment is urged.

Respectfully submitted,

WYETH

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